from normal rats. This finding is probably attributable to the lower levels of circulating testosterone in animals that can neither see nor smell[3]. Testosterone has an important stimulatory influence on normal renal growth[6].

Résumé. Chez les rats qui ont subi une ablation de la glande pinéale, la présence simultanée de perte de vue et d'anosmie empêchent la croissance compensatoire de l'ovaire chez la femelle et celle des glandes surrénales chez la mâle. Une hypertrophie du rein provoquée expérimentalement après une néphrectomie unilatérale n'est pas modifiée par la perte de vue et l'anosmie.

R. J. REITER

Department of Anatomy, The University of Texas Medical School at San Antonio, San Antonio (Texas 78229, USA), 13 March 1972.

Biological Properties of Synthetic Ser[4]-Arg[8]-Oxytocin (Ile[3]-Ser[4]-Arginine Vasopressin): Role of the Residue No. 4 in the Hormone-Pressor Receptor Interaction

The isolation of oxytocin and arginine vasopressin from the neurohypophysis of several mammals[1,2] has shown that these two nonapeptides, although structurally very similar (they differ only by 2 residues in positions 3 and 8) act on different receptors, since oxytocin is responsible for the uterotonic and milk-ejecting activities of the gland while vasopressin accounts for the pressor and antidiuretic activities. A hybrid molecule with the residue No. 3 of oxytocin (isoleucine) and the residue No. 8 of arginine vasopressin (arginine), called *arginine vasotocin*, has been synthesized and this peptide displayed on mammals the 4 activities mentioned above[3]. The role of residue No. 3 for the interaction with oxytocic receptor and that of residue No. 8 for the interaction with the pressor-antidiuretic receptor were thus disclosed.

More recently the identification of a new neurohypophysial hormone in bony fishes, *isotocin* (Ser[4]-Ile[8]-oxytocin) (ref.[4]) has shown that the substitution in position 4 does not decrease strongly the rat oxytocic activity of the nonapeptide, a result in agreement with some data previously obtained with synthetic 4-substituted analogues of oxytocin[5]. Furthermore, synthetic Thr[4]-oxytocin is about twice as active as natural oxytocin[6]. Except the residues 4 and 8 which can be substituted, the other amino acids of the molecule seem necessary for the oxytocic activity although the α amino group can be removed[7].

About the amino acids involved in the pressor activity, which is associated with the antidiuretic activity, it has long been known that a basic residue (arginine, lysine, ornithine, diaminobutyric acid) is necessary in position 8 (ref.[5]). Only a few analogues of vasopressin with substitutions in other positions have so far been synthesized, so that the relative importance of these positions, in particular that of position 4, is poorly known. Ser[4]-Arg[8]-oxytocin (or Ile[3]-Ser[4]-arginine vasopressin) has recently been synthesized[8] and a study of some of its biological properties has now been carried out.

From another point of view, because Ser[4]-Arg[8]-oxytocin has a serine residue in position 4 like hormones found in fishes (such as isotocin of teleosts[4] or glumitocin of rays[9]) and an arginine residue in position 8 like arginine vasotocin found in all non-mammalian vertebrates, this peptide might be a common evolutionary precursor of these hormones in a very primitive species. Isotocin and arginine vasotocin are simultaneously present not only in Neopterygii[4,10,11] but also in some Paleopterygii such as Polypterus[12]; on the other hand all the cartilaginous fishes have at least 2 neurohypophysial hormones[9,13]. If it is assumed that a gene duplication has occurred in early vertebrates for giving 2 similar peptides in fishes and higher classes, a single peptide might be found for instance in some species of the primitive class of Cyclostomata, and Ser[4]-Arg[8]-oxytocin might be a possible candidate for this single neurohypophysial hormone.

Table I. Amino acid composition of synthetic Ser[4]-Arg[8]-oxytocin (number of residues per mole, aspartic acid being taken as reference)

Amino acid	Theoretical values	Oxidized sample		Reduced sample	
		46 nmol	38 nmol	32 nmol	52 nmol
Asp	1	1.00	1.00	1.00	1.00
Ser	1	0.89	0.90	0.95	0.85
Pro	1	1.11	1.53	1.09	0.78
Gly	1	1.03	0.92	1.10	1.09
Ile	1	1.02	1.01	0.99	1.03
Tyr	1	0.68	0.42	1.04	0.92
Arg	1	1.00	0.89	0.95	0.97
Cys	2	2.40	2.11	—	0.67

	1	2	3	4	5	6	7	8	9
Isotocin	Cys	Tyr	Ile	Ser	Asn	Cys	Pro	Ile	Gly(NH_2)
Vasotocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Arg	Gly(NH_2)
Ser[4]-Arg[8]-oxytocin	Cys	Tyr	Ile	Ser	Asn	Cys	Pro	Arg	Gly(NH_2)

Amino acid sequences of isotocin, arginine vasotocin and Ser[4]-Arg[8]-oxytocin

1 V. DU VIGNEAUD, Harvey Lect, Ser. E, *1* (1954–1955).
2 R. ACHER and C. FROMAGEOT, Ergebn. Physiol. *48*, 286 (1955).
3 P. G. KATSOYANNIS and V. DU VIGNEAUD, J. biol. Chem. *233*, 1352 (1958).
4 R. ACHER, J. CHAUVET, M. T. CHAUVET and D. CREPY, Biochim. biophys. Acta *58*, 624 (1962).
5 B. BERDE and R. A. BOISSONNAS, in *The Pituitary Gland* (Eds. G. W. HARRIS and B. T. DONOVAN; Butterworths, London 1966), vol. 3, p. 624.
6 M. MANNING, E. COY and W. H. SAWYER, Biochemistry *9*, 3925 (1970).
7 V. DU VIGNEAUD, G. WINESTOCK, V. V. S. MURTI, D. B. HOPE and R. D. KIMBROUGH JR., J. biol. Chem. *235*, PC 64 (1960).
8 A. JÖHL, unpublished results.
9 R. ACHER, J. CHAUVET, M. T. CHAUVET and D. CREPY, Biochim. biophys. Acta *107*, 393 (1965).
10 R. ACHER, J. CHAUVET, M. T. CHAUVET and D. CREPY, Comp. Biochem. Physiol. *14*, 245 (1965).
11 R. ACHER, J. CHAUVET, M. T. CHAUVET and D. CREPY, Gen. comp. Endocr. *11*, 535 (1968).
12 R. ACHER, J. CHAUVET and M. T. CHAUVET, FEBS Lett. *11*, 332 (1970).
13 R. ACHER, J. CHAUVET and M. T. CHAUVET, C. r. Soc. Biol. Paris *274*, 313 (1972).

Table II. Molecular activities of some 4- or (and) 8-substituted analogues of oxytocin[a]

Amino acids in positions 3, 4 and 8	Trivial names	Rat oxytocic activity	Chicken depressor activity	Rat pressor activity
Ile^3-Gln^4-Leu^8	Oxytocin	450 ± 30	450 ± 30	5 ± 1
Ile^3-Ser^4-Leu^8		190 ± 30	220 ± 20	<0.1
Ile^3-Ser^4-Ile^8	Isotocin	145 ± 12	310 ± 15	0.6 ± 0.01
Ile^3-Ser^4-Gln^8	Glumitocin	7.8 ± 0.6	—	0.4 ± 0.1
Ile^3-Gln^4-Arg^8	Arg-vasotocin	120 ± 16	300 ± 42	255 ± 16
Ile^3-Ser^4-Arg^8		66 ± 15	311 ± 28	20.2 ± 3.4
Ile^3-Gln^4-Lys^8		80 ± 10	215 ± 3	133 ± 13
Phe^3-Gln^4-Lys^8	Lys-vasopressin	5 ± 0.5	42 ± 5	285 ± 21
Phe^3-Ser^4-Lys^8		0.9 ± 0.2	10 ± 0.7	3.3 ± 0.5

[a] Values for synthetic substances, taken from BERDE and BOISSONNAS[5, 20] except for glumitocin and Ser^4-Arg^8-oxytocin (present communication).

A sample of synthetic Ser^4-Arg^8-oxytocin (Figure), kindly supplied by Dr. JÖHL, was used for the experiments. Amino acid analysis of the peptide was carried out according to SPACKMAN et al.[14] either with performic acid-oxidized samples or β-mercaptoethanol-reduced samples. Hydrolysis is performed with 6 *N* HCl for 48 h at 105°C in evacuated sealed tubes. The results are given in Table I.

Rat oxytocic[15], chicken depressor[16] and rat pressor[17] activities have been measured and molecular activities (u. U.S.P./μmole) have been determined. Table II shows the values for Ser^4-Arg^8-oxytocin as well as those previously found for some analogues with substitutions in positions 4 or (and) 8, given for comparison.

Ser^4-Arg^8-oxytocin has an oxytocic activity about half that of arginine vasotocin but its pressor activity is about 12 times weaker. The substitution of serine for glutamine in position 4 greatly affects the latter activity. This finding is in agreement with the dramatic decrease of the pressor activity when serine replaces glutamine in lysine vasopressin (Table II). Although this is merely an interchange between 2 polar, neutral, short side-chain residues, there is a strong effect on pressor activity in contrast to that observed for oxytocic activity.

Oxytocic activity with addition of magnesium[18] has also been determined; the ratios of 3 biological properties to oxytocic activity without magnesium have been calculated for Ser^4-Arg^8-oxytocin and compared to those found for chicken arginine vasotocin[19] (Table III).

It can be seen that substitution in position 4 strongly alters the 'pharmacological profile' of arginine vasotocin and the distinction between this hormone and Ser^4-Arg^8-oxytocin is relatively easy on the basis of biological properties.

Bioassays previously made with analogues of lysine vasopressin in which the phenylalanine residue No. 3 is replaced by serine, tyrosine or tryptophan have shown that pressor activity is virtually abolished while the replacement by the hydrophobic aliphatic isoleucine leads to a decrease of about 50% only[5, 20]. The substitution of glutamine in position 4 by alanine or serine destroys the pressor activity as well as the substitution of asparagine in position 5 by serine[5, 20].

Apparently no position is largely 'open' to mutations with maintenance of the pressor activity. The strict fit to specific receptor might explain the great stability of arginine vasotocin in the course of the evolution of non-mammalian vertebrates and of arginine vasopressin in the evolution of mammals. The switch of arginine vasotocin to arginine vasopressin[21] has involved a single substitution in position 3 (phenylalanine for isoleucine). The consequence is the disappearance of the strong secondary oxytocic activity of the molecule and an increase of the pressor-antidiuretic property, that means a better specialization of the hormone.

Résumé. Les propriétés pharmacologiques de la Ser^4-Arg^8-ocytocine (Ser^4-vasotocine) synthétique ont été

[14] D. H. SPACKMAN, W. H. STEIN and S. MOORE, Analyt. Chem. *30*, 1190 (1958).
[15] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[16] J. M. COON, Archs int. Pharmacodyn *62*, 79 (1939).
[17] R. N. LANDGREBE, M. H. F. MACAULAY and H. WARING, Proc. R. Soc. Edinburgh, B *62*, 202 (1946).
[18] R. A. MUNSICK, Endocrinology *66*, 451 (1960).
[19] R. ACHER, J. CHAUVET and M. T. CHAUVET, Europ. J. Biochem. *17*, 509 (1970).
[20] B. BERDE and R. A. BOISSONNAS, in *Handbook of Experimental Pharmacology* (Ed. B. BERDE; Springer-Verlag, Berlin, Heidelberg 1968), vol. 23, p. 802.
[21] R. ACHER, in *Biochemical Evolution and the Origin of Life* (Ed. E. SCHOFFENIELS; North Holland Co., Amsterdam 1971).

Table III. Comparison of the ratios of pharmacological activities of Ser^4-Arg^8-oxytocin and Arg^8-oxytocin (arginine vasotocin)[a]

Peptide	OMg^{++}/O	P/O	D/O
Gln^4-Arg^8-oxytocin (vasotocin)	2.12 ± 0.28	1.64 ± 0.41	2.50 ± 0.30
Ser^4-Arg^8-oxytocin	4.83 ± 2.06	0.29 ± 0.05	4.91 ± 0.39

[a] O, rat oxytocic activity without magnesium[15]; OMg^{++}, rat oxytocic activity with magnesium[18]; P, rat pressor activity[17]; D, chicken depressor activity[16]. Values for arginine vasotocin are taken from reference[19].

étudiées. La substitution en position 4 de la glutamine par la sérine diminue considérablement l'activité pressique de la vasotocine, ce qui montre l'importance de cette position dans les interactions entre les hormones neurohypophysaires et le récepteur vasopressique.

JACQUELINE CHAUVET, MARIE-THÉRÈSE CHAUVET and R. ACHER[22]

Laboratoire de Chimie Biologique, Université de Paris VI, 96, Boulevard Raspail, F–75 Paris 6e (France),
29 May 1972.

[22] The authors are indebted to Dr. ALBERT JÖHL (Ciba-Geigy Laboratories) for a sample of synthetic Ser[4]-Arg[8]-oxytocin. They thank Mrs. CHRISTINE CAULLIER for ther skilled technical assistance.

Chromosome Replication in Cells of a Continuous Line Derived from *Aedes albopictus* (Skuse) Larvae

Over the past several years continuous cell lines derived from a number of mosquito species have been developed[1-9]. Although there is some data on the chromosome complement of some of these lines[1,5,7,9,10], it appears valuable to have the karyological characterization of some of these strains as complete as possible. In previous publications, we have reported data on the karyology of 3 cell lines derived from either *Aedes aegypti* or *Aedes albopictus*[10], and on the pattern of constitutive heterochromatin distribution in tissue culture cells from the latter species[11]. This paper will give information on the pattern of DNA synthesis by the chromosome complement of the *A. albopictus* cell line.

Studies were performed in passage 162 of a cell line (designated ATC-15) initiated from first instar larvae of the mosquito *A. albopictus* (Skuse) by SINGH[2] and grown on the medium described by him. Cultures in the log phase of growth were divided into 2 groups and respectively treated for 5 or 3 h with 1 μC/ml of 3HTdR (specific activity 6.9 C/m*M*). Both groups of cultures received 0.12 μg/ml of Colcemid 3 h before harvesting. Cells were removed by trypsinization, hypotonically treated and fixed in 3:1 ethanol:acetic acid. Chromosome spreads were obtained by air drying and stained with carbol fuchsin. Slides were mounted with AR10 Kodak stripping film and exposed for 10 days. Autoradiograms were processed and analyzed as described elsewhere[12].

The karyology of ATC-15 cells has been recently reported[10,11], hence, it will be only briefly mentioned here. The chromosome complement is formed by 3 pairs of metacentric chromosomes. Following the usual nomenclature for mosquito chromosomes the longest and shortest pairs in the set are numbered 3 and 1, respectively. Approximately 48% of cells had chromosome aberrations ranging from chromatid gaps to open or rearranged chromosomal breakages. These aberrations were not random, but preferentially located in the proximal third of 1 arm in pair 1 and in the distal third of 1 arm in pairs 2 and 3. The incidence of polyploidy varied from 10 to 18%[10].

Autoradiograms obtained from the 5 and 3 h 3HTdR treatment showed 82 and 48% of labeled mitosis, respectively. Thus, a G2 period of approximately 3 h may be assumed for ATC-15 cells[13]. The amount of silver grains on labeled metaphases was variable and allowed one to assemble the cells in a series of continuous decreasing radioactivity. Metaphases at the beginning of the series showed labeling all over the chromosome complement (Figure 1). In further stages the deposition of silver grains on the chromosomes decreased and the absence of labeling in the pericentromeric areas of pairs 2 and 3, and occasionally, in the middle third of 1 arm in pair 1 was noticed (Figures 2 and 3). Afterwards, unlabeled areas increased in extent and in metaphases at the end of the series labeling was restricted to: a) the upper half of one arm and the distal third of the other in pair 3; b) the distal half of both arms in pair 2; c) the distal third of one arm and the proximal third of the other in pair 1; d) centromere of the 3 pairs (Figures 4 and 5). Patterns c) and d) were less constant and conspicuous than patterns a) and b).

The heaviest labeled metaphases arose mainly from cultures treated with 3HTdR for 5 h. On the other hand most of the metaphases with less than half of the complement labeled stemmed from the 3 h treatment. In cells continuously treated with 3HTdR unlabeled chromosome areas represent the genome regions which have finished replication before the isotope was added. Thus, by treating different cell populations for variable time lapses it is possible to obtain a sequence of labeling patterns representing various stages of the S phase. In the foregoing series, complete labeled metaphases probably arose from intermediate stages of the S period in which most or all replicating units in the genome are engaged in DNA synthesis. On the other hand, radioactive regions from partly labeled metaphases illustrate the areas involved in replication during the late and final stages of the S period (late replicating regions).

It is presently held that late replication is one of the most conspicuous properties of heterochromatin[14]. Ac-

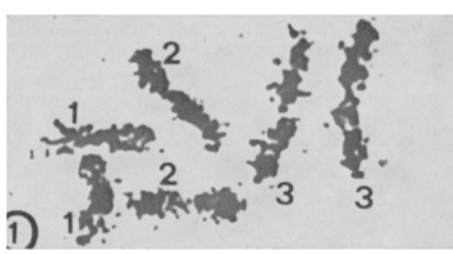

Fig. 1. Autoradiogram showing labeling all over the chromosome complement. Chromosomes are numbered. All photographs × 1,500.

[1] T. D. C. GRACE, Nature, Lond. *211*, 366 (1966).
[2] K. R. P. SINGH, Curr. Sci. *36*, 506 (1967).
[3] J. PELEG, J. gen. Virol. *5*, 463 (1969).
[4] M. G. R. VARMA and M. PUDNEY, J. med. Ent. *6*; 432 (1969).
[5] B. H. SWEET and J. S. MCHALE, Expl. Cell Res. *61*; 51 (1970).
[6] U. K. M. BHAT and K. R. P. SINGH, Curr. Sci. *39*, 388 (1970).
[7] I. SCHNEIDER, J. Cell. Biol *42*, 603 (1969).
[8] M. PUDNEY and M. G. R. VARMA, Expl. Parasit. *29*, 7 (1971).
[9] S. H. HSU, W. H. MAO and J. H. CROSS, J. med. Ent. *7*, 703 (1970).
[10] N. O. BIANCHI, B. H. SWEET and J. AYRES, Proc. Soc. exp. Biol. Med. *140*, 130 (1972).
[11] N. O. BIANCHI, B. H. SWEET and J. AYRES, Expl. Cell Res. *69*, 236 (1971).
[12] N. O. BIANCHI, A. LIMA-DE-FARIA and H. JAWORSKA, Hereditas *31*, 207 (1964).
[13] J. E. SISKEN, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT, Academic Press, New York 1964), p. 387.
[14] A. LIMA-DE-FARIA, in *Handbook of Molecular Cytology* (Ed. A. LIMADE-FARIA, American-Elsevier, New York 1969), p. 278.